

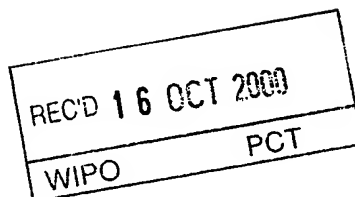


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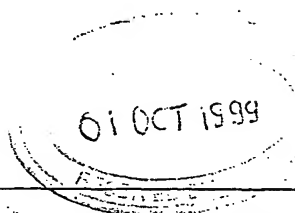
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SmithKline Beecham plc
New Horizons Court, Brentford, Middx TW8 9EP,
Great Britain

Patents ADP number (*if you know it*)

If the applicant is a corporate body, give the country/state of its incorporation

5776299002

TS

4. Title of the invention

Novel Transgenic Animal

5. Name of your agent (*if you have one*)

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Novel Transgenic Animal

The present invention relates to a transgenic animal expressing the human uncoupling protein 3 (UCP3) gene and to the uses thereof. Furthermore the invention relates to methods for constructing the transgenic animal and to the transgenes used in this construction.

With the advent of classical transgenesis and embryonic stem (ES) cell technology in the past two decades, the house mouse (*Mus musculus*) has rapidly become the mammalian model system of choice for the study of gene function. The mouse genome can be manipulated in several ways:

1. Introduction of a "transgene" either randomly (pronuclear injection) or into pre-determined locus (via ES cells).
2. Targeted disruption ("knockout") of the gene of interest.
3. Targeted mutagenesis of the endogenous gene ("knock-in").

1. Introduction of a transgene into the mouse genome.

In general, transgenic mice expressing transgenes represent gain-of-function mutations. Loss-of-function mutations are usually obtained by gene targeting. However, overexpression of a dominant negative mutant protein may result in the functional knockout of the gene of interest (e.g. Stacey et al., *Nature* 332, 131, 1988). Most commonly, transgenesis is used for the study of tissue- and developmental stage-specific gene regulation, for experiments of the phenotypic effects of transgene expression or for the creation of mouse models of human disease, in particular neurodegenerative conditions like Alzheimer's disease, Huntington's disease, motor neuron disease etc (for general review see R. Jaenisch, *Science* 240, 1468, 1988). Typically, the transgenic construct is microinjected into the male pronucleus of fertilised eggs (Gordon and Ruddle, *Science* 214, 1244, 1981), resulting in the random integration into one locus of a varying number of copies, usually in a head to tail array (Costantini and Lacy, *Nature* 294, 92, 1981). Alternatively, transgenes can be introduced via ES cells, using electroporation, retroviral vectors or lipofection for gene transfer. This has been successfully demonstrated for a number of very large, BAC or YAC derived transgenes (Hodgson et al., *Neuron* 23, 181, 1999; Lamb et al., *Nature Neuroscience* 2, 695, 1999). Due to positional effects, expression of a randomly integrated transgene may be inhibited or occur in a non-authentic manner (with respect to the chosen promoter). To overcome these potential problems transgenes can be inserted into pre-determined loci (ROSA26, HPRT etc.) that support transcriptional activity and whose disruption by the insertional mutagenesis is without consequences (Zambrowicz et al., *Proc Natl Acad Sci USA* 94, 3789, 1997; P. Soriano, *Nature Genetics* 21, 70, 1999). Again, this technology is ES cell based

and essentially a special case of gene knock-in (see below). Transgenes can also be expressed in other rodents, for example rats (e.g. Breban et al., *J Immunol* **156**, 794, 1996; Gariepy et al., *J Clin Invest* **102**, 1092, 1998). In this case the transgene is introduced into the animal by pronuclear injection as the ES cell route is currently only
5 available for mice.

2. Targeted disruption ("knockout") of the gene of interest.

Gene knockout involves the conversion of the gene of interest into a null allele, thus completely disrupting the function of the gene (Joyner AL (editor) *Gene targeting: A practical approach*. IRL Press, Oxford, England, 1993). Analysis of the resulting
10 phenotype may then allow conclusions as to the function of the gene product. This technology is based on the homologous recombination in embryonic stem cells of a suitable targeting vector with the endogenous gene. Typically, a positive-negative selection strategy is used to enrich for ES cell clones that have undergone the desired
15 recombination event (Thomas and Capecchi, *Cell* **51**, 503, 1987; Soriano et al., *Cell* **64**, 693, 1991). In most cases this results in the replacement of essential coding sequences by foreign DNA (usually a positive selection marker). In a second step the latter is then removed by a site-specific recombinase (Abremski et al., *J Biol Chem* **261**, 391, 1986).
Over the last several years tremendous progress has been made to gain spatio-temporal
20 control over the knockout event ("conditional" or "inducible" gene targeting; Rossi and Blau, *Curr Opin Biotechnol* **9**, 451, 1998; A Porter, *TIG* **14**, 73, 1998) but none of the technologies employed has so far yielded entirely satisfactory results. Most avenues toward regulated gene knockout rely on the activity of site-specific recombinases (Cre- or Flp recombinase) that recognize short inverted repeats (LoxP or FRT sites, respectively)
25 and excise the stretch of DNA that is flanked by these repeats (for example "floxed" 5' exon of target gene). Temporal control over recombinase activity (Schwenk et al., *Nuc Acids Res* **26**, 1427, 1998; Mansuy et al., *Neuron* **21**, 257, 1998) thus translates into inducibility of gene targeting (Rossant and McMahon, *Genes and Development* **13**, 142, 1999 (meeting review)). However, a significant disadvantage of recombinase-based
30 approaches is the irreversibility of the knockout.

3. Targeted mutagenesis of the endogenous gene ("knock-in").

Gene knock-in is based on the same principle of homologous recombination as gene knockout. However, a gene knock-in is not designed to ablate the function of the gene but to introduce changes in the coding (or in some instances intron) sequence that are intended to alter the function of the endogenous gene. This may range from a single
 5 nucleotide exchange (point mutation; e.g. Cho et al., Science **279**, 867, 1998) to the deletion (or addition) of sequences that encode functional protein domains (e.g. Sprengel et al, Cell **92**, 279, 1998) or the swapping of the entire mouse coding sequence with, for example, the human cDNA. The latter manipulation is often employed in a pharmaceutical setting, in support of drug development, since some compounds exhibit
 10 antagonist or agonist properties against the human but not the murine target.

Mitochondrial uncoupling proteins (UCPs) are inner mitochondrial membrane proteins whose function is to uncouple mitochondrial respiration from ADP
 15 phosphorylation (see Ricquier *et al* (1999) J Intern Med 245(6):637-42 for review).

The first member of the family, mitochondrial uncoupling protein 1 (UCP1; Bouillaud et al (1985) Proc Natl Acad Sci 82(2) p445-448; Jacobsson et al (1985) J. Biol. Chem. 260(30) p16250-16254), is expressed exclusively in the brown adipocyte. It functions to uncouple mitochondrial respiration by dissipating the mitochondrial proton
 20 gradient, normally used to drive ATP synthesis, to produce heat as a consequence of fatty acid oxidation. In rodents brown adipose tissue contributes to cold adaptation and body weight regulation via non-shivering thermogenesis and diet-induced thermogenesis respectively. However, since little brown adipose tissue (BAT) is present in adult humans, UCP1 is unlikely to play a major role in either of these important homeostatic
 25 functions and although many rodentian tissues display a mitochondrial proton leaks that may subserve these functions the precise molecular mechanism by which these leaks occur are not known. The recent discovery of uncoupling protein homologues with wider tissue distribution in both animals and humans may provide some insight into non-shivering and diet-induced thermogenesis in humans.

30 The second member of the uncoupling protein family, uncoupling protein-2, (UCP2) was reported independently by Fleury et al. (Nature Genetics **15**, 269, 1997) and Gimeno, et al. (Diabetes **46**, 900-906, 1997). UCP2 shares 59% identity to UCP1 at the

amino acid level. However, unlike UCP1, UCP2 is more widely expressed in human tissues predominantly in white adipose tissue, skeletal muscle (a major site of fuel utilisation and thermogenesis) and components of the immune system. The varying level of expression of UCP2 in mouse strains with differential susceptibility to weight gain is consistent with it playing some role in weight gain potential (Fleury et al. 1997 supra). In mice, UCP2 maps close to a quantitative trait locus (QTL) on chromosome 7 associated with obesity. Human UCP2 has been mapped to the homologous region of the long arm of chromosome 11 (Bouchard et al., Human Molecular Genetics 6, 1887-1889, 1997; Solanes et al., J.Biol.Chem 272 25433-25436, 1997).

10 Shortly after the publication of the sequence for UCP2 a third member of the uncoupling protein family was identified and termed UCP3 (WO98/39432 (SmithKline Beecham); Boss et al., FEBS lett 408 39-42, 1997; Vidal-Puig et al., Biochem.Biophys.Res.Commun. 235 79-82, 1997). UCP3 is 73% identical to UCP2 and 59% identical to UCP1 at the amino acid level. In contrast to the wide tissue distribution of UCP2, UCP3 mRNA is predominantly expressed in skeletal muscle. Skeletal muscle is an important site for resting metabolic rate and UCP3 levels in skeletal muscle may be a determinant of energy expenditure and metabolic efficiency in Pima Indians (Schrauwen et al., Diabetes 48 146-149, 1999). UCP3 also maps to 11q13 and is adjacent to UCP2 to within 100 kb (Gong et al., Biochem.Biophys.Res.Commun. 256 27-32, 1997; Solanes et al., 1997 supra) suggesting that they are evolutionarily very close. UCP3 has also been implicated in wound healing (SmithKline Beecham plc patent application no. PCT/EP98/07802).

25 There is a need to characterise further the UCP genes and the UCP polypeptides expressed therefrom, to determine the function of the polypeptides and to investigate the effect of increased or reduced expression and its relevance to disease. In addition the consequences of altered spatial or temporal expression of the UCP polypeptide need to be investigated as well as the effects of altered UCP polypeptides, where such alterations may have arisen through mutation for example. There is also a need to provide a means to identify and evaluate (with regard efficacy and safety) chemical compounds that modulate the activity of the UCP genes or polypeptides. Such modulation may, for example, be afforded by compounds which bind to and activate (agonist) or inhibit activation

30

(antagonist) of the UCP polypeptide. Compounds identified thereby could be useful in therapy of diseases where inappropriate expression of the UCP polypeptide or a mutated form of the UCP polypeptide is implicated. There is a particular need to characterise further the human UCP3 polypeptide, because of its potential role in wound healing. One method for facilitating such studies is the generation and use of a transgenic rodent capable of expressing the human UCP3 polypeptide.

The present invention provides a transgenic rodent whose genome comprises a polynucleotide encoding a polypeptide which has at least 95% identity to the human mitochondrial uncoupling protein-3 (UCP3) of SEQ ID NO:2 under the control of a regulatory sequence facilitating expression of said polypeptide. In a preferred aspect the transgenic rodent is a mouse or rat, preferably a mouse.

Preferably the encoded polypeptide comprises a polypeptide having the amino acid sequence of SEQ ID NO:2. More preferably the polypeptide has the amino acid of SEQ ID NO:2.

Preferably the polypeptide is encoded by a polynucleotide having at least 95% identity to the polynucleotide sequence of SEQ ID NO:1. More preferably the polypeptide is encoded by the polynucleotide of SEQ ID NO:1. In a further embodiment the polypeptide may be expressed from a polynucleotide, which, as a result of the redundancy (degeneracy) of the genetic code, also encodes the polypeptide of SEQ ID NO:2.

The assembly of a transgenic construct follows standard cloning techniques, that are well known in the art (for example see Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)). The cDNA to be overexpressed can be prepared from a mRNA extracted from a relevant tissue, preferably a tissue in which the protein of interest is known to be expressed. The cDNA, along with the promoter of choice and other components such as artificial introns and reporter genes, can then be inserted into a cloning vector by restriction digest and ligation. Suitable cloning vectors for the assembly of transgenes provide for acceptable yields of DNA. Vectors such as pBluescript are particularly preferred as in addition to good yield, they provide desirable unique restriction sites flanking the transgene (for example BssHII in pBluescript) for convenient removal of the

vector portion of the construct prior to pronucleus injection. Should any of the components of the transgene inserted into the vector contain within their sequence additional restriction sites that are identical to the flanking restriction sites, such restriction sites will clearly not be unique and so alternative restriction sites must be identified or
5 introduced for effective vector removal without transgene disruption.

In a further embodiment the transgene can be isolated from genomic DNA.

The expression of the transgene in the host genome may be controlled temporally and/ or spatially by placing the transgene under the control of an appropriate regulatory sequence, for example a promoter. The choice of promoter depends on the biological
10 question that the mouse model is intended to answer. Most transgenes contain tissue-specific promoters that, in the best case scenario, lead to a spatially and temporally authentic (with respect to the endogenous gene) expression pattern of the transgene. Other promoters provide for ubiquitous expression across the entire organism. One example of a tissue specific promoters is the alpha-actin promoter that drives transcription in skeletal
15 muscle. There are many other tissue-specific promoters that can be used. Such promoters include, but are not limited to, the calcium-calmodulin dependent Kinase II (CamKII) promoter for expression in neurons and neurosecretory cells; albumin promoter for hepatocytes; insulin promoter for pancreatic beta cells; rhodopsin promoter for retinal rods and cones; myogenin promoter for skeletal muscle; promoters of certain keratins for
20 dermis; etc.

Regulatory sequences, such as promoters, are operably linked to the coding sequence of the gene of interest in a manner that will permit the required temporal and spatial expression of the transgene. Methods of so linking regulatory sequences to cDNAs to facilitate their expression are widely known in the art. Such methods include
25 directly ligating a polynucleotide sequence comprising a regulatory sequence to the coding region of the transgene. Additional polynucleotide sequences may be included that modulate expression in the required manner. Examples of additional sequences include enhancer elements, artificial introns and others. In addition the nucleotide sequence of a known promoter, or other regulatory sequence, may be modified to increase
30 levels of expression. Such modifications can be achieved using, for example, site-directed mutagenesis methods well known in the art (see Sambrook et al, *supra*).

In addition to modifying the sequence of regulatory elements to enhance, or otherwise change, expression levels, the coding sequence of the gene of interest may be modified to enhance or otherwise affect expression levels. For example if the transgene is from a different species than the host, the codon usage of the transgene can be altered to match more closely that of the host. It is well known in the art that different organisms use the 64 coding and stop codons at different frequencies. Codons that are infrequently used in an organism are termed "rare codons". If a transgene includes a codon that is a rare codon in the host, expression levels may be severely reduced. One solution is to replace one or more rare codons in the transgene with codons that are frequently used in the host. Other modifications to the transgene sequence include modifying the polynucleotide sequence surrounding the start codon (the initiator methionine encoding codon) to make this more closely match the consensus "Kozak" sequence (A/G CCATGG, where the ATG in bold is the start codon; see for example Kozak, M., *Nucleic Acids Res* (1984) May 11;12(9):3873-3893)). In the transcribed mRNA molecule the Kozak sequence is believed to provide the optimal environment for initiation of translation of the polypeptide.

Preferably, prior to the introduction of the transgene into the host cell, the vector portions are removed by restriction enzyme digestion, for example by using restriction sites in the vector that flank the transgene. Thus the genetic material that is actually introduced into the host cell will preferably comprise the coding sequence of the gene of interest and the regulatory sequences to which it has been operably linked together with other potential components of the transgene, for example a reporter gene. More preferably the genetic material will have only the transgene and the regulatory sequences to which it has been operably linked.

There are a number of techniques that permit the introduction of genetic material, such as a transgene, into the rodent germline. The most commonly used protocol comprises direct injection of the transgene into the male pronucleus of the fertilised egg (Hogan et al., *Manipulating the mouse embryo (A laboratory manual)* Second edition, CSHL Press 1994). The injected eggs are then re-transferred into the uteri of pseudo-pregnant recipient mothers. Some of the resulting offspring may have one or several copies of the transgene integrated into their genomes, usually in one integration site. These "founder" animals are then bred to establish transgenic lines and to back-cross into

the genetic background of choice. It is convenient to have the transgene insertion on both chromosomes (homozygosity) as this obviates the need for repeated genotyping in the course of routine mouse husbandry.

An alternative method to introduce the transgene into mice is the random
 5 insertion into the genome of pluripotent embryonic stem (ES) cells, followed by the
 —production of chimeric mice and subsequent germline transmission. Transgenes of up to
 several hundred kilobases of rodent DNA have been used to produce transgenic mice
 in this manner (for example Choi et al., *Nature Genet.* 4, 117-123 (1993); Strauss et al.,
Science 259, 1904-07 (1993)). The latter approach can be tailored such that the transgene
 10 is inserted into a pre-determined locus (non-randomly, for example ROSA26 or HPRT)
 that supports ubiquitous as well as tissue specific expression of the transgene (Vivian et
 al., *BioTechniques* 27, 154-162 (1999)).

The transgenic rodent is subsequently tested to ensure the required genotypic
 change has been effected. This can be done by, for example, detecting the presence of the
 15 transgene by PCR with specific primers, or by Southern blotting of tail DNA with a
 specific probe. Testing for homozygosity of the transgene insertion may be carried out
 using quantitative Southern blotting to detect a twofold difference in signal strength
 between hetero- and homozygous transgenic rodents. Once the desired genotype has been
 confirmed the transgenic rodent line is subjected to various tests to determine the gain-of-
 20 function phenotype. The tests involved in this phenotypic characterisation depend on
 what genotypic change has been effected, and may include, for example, morphological,
 biochemical and behavioural studies.

In a preferred aspect of the present invention the polynucleotide encoding the
 UCP3 polypeptide is predominantly expressed in skeletal muscle. Such near-exclusive
 25 expression may be facilitated by the transcriptional activity of skeletal muscle specific
 promoters. In a preferred embodiment the skeletal muscle-specific promoter is the alpha-
 actin promoter.

Phenotypic tests can be devised for examining the effect of overexpressing the
 human UCP3 gene. Such tests are based on the hypothesis that UCP3 protein uncouples
 30 the respiratory chain in the mitochondria from the generation of energy rich molecules
 (NADP, NADPH, and ultimately ATP, GTP), with the result that there is excess heat
 production. Thus, simple measurements like weight gain, food intake and body

temperature are preferred phenotypic tests for the initial analysis of the UCP3 transgenic rodents. Subsequently, glucose clearance and other parameters of glucose homeostasis can be investigated. In addition the time required for wound healing and general behavioural trends may be investigated. Based on the results of these studies, further
5 more specific tests can be devised to give a more detailed analysis of the consequences of UCP3 expression.

Although the primary function of such transgenic rodents is that of elucidating the function of a gene of interest, they may also be used in the validation of the
10 polypeptide expressed from the transgene as a drug target. Transgenic rodents can also be used to test the efficacy of a drug and a drug administration regime. Once established, these transgenic rodents could be used to investigate the effects of various drug treatments on the course of the disease (in the animal model setting). In addition, transgenic overexpression models may produce surprising, unexpected results by way of the
15 resulting phenotype. This could result in the identification of "new" disease indications, or serve as a warning, for example when the transgenic rodents display developmental abnormalities or develop tumours.

The following definitions are provided to facilitate understanding of certain terms used
20 frequently hereinbefore.

A "Transgene" comprises a polynucleotide, isolated from nature, which has been manipulated *in-vitro* and subsequently introduced into the genome of the same or a different species in either the native or modified forms, such that it is stably and heritably maintained in that genome. Native forms include unmodified polynucleotides isolated
25 from a source different to that into which it is subsequently introduced, for example a human polynucleotide sequence introduced into a mouse genome. Modified polynucleotides include those which have one or more nucleotide substitutions, deletions, insertions or inversions. Native or modified polynucleotides may be operably linked to a heterologous promoter, or other regulatory sequence, from a different gene within the
30 same species or from a gene in a different species. A polynucleotide is operably linked to a regulatory sequence when, for example, it is placed under the transcriptional control of said regulatory sequence. The polynucleotide may or may not encode a polypeptide, and

if a polypeptide is expressed from the polynucleotide, said polypeptide may or may not be full-length relative to that encoded by the original polynucleotide isolated. The term transgene is generally used to refer to the polynucleotide and the regulatory sequences to which it is operably linked.

5 An organism into which a transgene has been introduced is termed a "transgenic" organism.

"Regulatory sequences" refer to DNA or RNA polynucleotide sequences, which are usually non-coding, that are involved in the regulation of transcriptional activity or tissue-specific enhancement or silencing of gene transcription. Such regulatory sequences
10 include promoters and enhancers.

"Identity" as known in the art, is a relationship between two or more polypeptide sequences or two or more polynucleotide sequences, as determined by comparing the sequences. In the art, "identity" also means the degree of sequence relatedness between polypeptide or polynucleotide sequences, as the case may be, as determined by the match
15 between strings of such sequences. "Identity" and "similarity" can be readily calculated by known methods, including but not limited to those described in (*Computational Molecular Biology*, Lesk, A.M., ed., Oxford University Press, New York, 1988; *Biocomputing: Informatics and Genome Projects*, Smith, D.W., ed., Academic Press, New York, 1993; *Computer Analysis of Sequence Data*, Part I, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; *Sequence Analysis in Molecular Biology*, von Heinje, G., Academic Press, 1987; and *Sequence Analysis Primer*, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; and Carillo, H., and Lipman, D., *SIAM J. Applied Math.*, 48: 1073 (1988). Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine
25 identity and similarity are codified in publicly available computer programs. Preferred computer program methods to determine identity and similarity between two sequences include, but are not limited to, the GCG program package (Devereux, J., et al., *Nucleic Acids Research* 12(1): 387 (1984)), BLASTP, BLASTN, and FASTA (Altschul, S.F. et al., *J. Molec. Biol.* 215: 403-410 (1990)). The BLAST X program is publicly available
30 from NCBI and other sources (*BLAST Manual*, Altschul, S., et al., NCBI NLM NIH Bethesda, MD 20894; Altschul, S., et al., *J. Mol. Biol.* 215: 403-410 (1990)). The well known Smith Waterman algorithm may also be used to determine identity.

Preferred parameters for polypeptide sequence comparison include the following:

1) Algorithm: Needleman and Wunsch, J. Mol Biol. 48: 443-453 (1970)

Comparison matrix: BLOSSUM62 from Hentikoff and Hentikoff, Proc. Natl. Acad. Sci. USA. 89:10915-10919 (1992)

5 Gap Penalty: 12

Gap Length Penalty: 4

A program useful with these parameters is publicly available as the "gap" program from Genetics Computer Group, Madison WI. The aforementioned parameters are the default parameters for peptide comparisons (along with no penalty for end gaps).

10 Preferred parameters for polynucleotide comparison include the following:

1) Algorithm: Needleman and Wunsch, J. Mol Biol. 48: 443-453 (1970)

Comparison matrix: matches = +10, mismatch = 0

Gap Penalty: 50

Gap Length Penalty: 3

15 Available as: The "gap" program from Genetics Computer Group, Madison WI. These are the default parameters for nucleic acid comparisons.

By way of example, a polynucleotide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:1, that is be 100% identical, or it may include up to a certain integer number of nucleotide alterations as compared to the reference sequence. Such alterations are selected from the group consisting of at least one nucleotide deletion, substitution, including transition and transversion, or insertion, and wherein said alterations may occur at the 5' or 3' terminal positions of the reference nucleotide sequence or anywhere between those terminal positions, interspersed either individually among the nucleotides in the reference sequence or in one or more contiguous groups within the reference sequence. The number of nucleotide alterations is determined by multiplying the total number of nucleotides in SEQ ID NO:1 by the numerical percent of the respective percent identity (divided by 100) and subtracting that product from said total number of nucleotides in SEQ ID NO:1, or:

$$n_n \leq x_n - (x_n \cdot y),$$

30 wherein n_n is the number of nucleotide alterations, x_n is the total number of nucleotides in SEQ ID NO:1, and y is, for instance, 0.70 for 70%, 0.80 for 80%, 0.85 for 85%, 0.90 for 90%, 0.95 for 95%, etc., and wherein any non-integer product of x_n and y is rounded

down to the nearest integer prior to subtracting it from x_n . Alterations of a polynucleotide sequence encoding the polypeptide of SEQ ID NO:2 may create nonsense, missense or frameshift mutations in this coding sequence and thereby alter the polypeptide encoded by the polynucleotide following such alterations.

- 5 Similarly, a polypeptide sequence of the present invention may be identical to the reference sequence of SEQ ID NO:2, that is be 100% identical, or it may include up to a certain integer number of amino acid alterations as compared to the reference sequence such that the % identity is less than 100%. Such alterations are selected from the group consisting of at least one amino acid deletion, substitution, including conservative and
10 non-conservative substitution, or insertion, and wherein said alterations may occur at the amino- or carboxy-terminal positions of the reference polypeptide sequence or anywhere between those terminal positions, interspersed either individually among the amino acids in the reference sequence or in one or more contiguous groups within the reference sequence. The number of amino acid alterations for a given % identity is determined by
15 multiplying the total number of amino acids in SEQ ID NO:2 by the numerical percent of the respective percent identity (divided by 100) and then subtracting that product from said total number of amino acids in SEQ ID NO:2, or:

$$n_a \leq x_a - (x_a \cdot y),$$

- wherein n_a is the number of amino acid alterations, x_a is the total number of amino acids
20 in SEQ ID NO:2, and y is, for instance 0.70 for 70%, 0.80 for 80%, 0.85 for 85% etc., and wherein any non-integer product of x_a and y is rounded down to the nearest integer prior to subtracting it from x_a .

Examples

Example 1 – Preparation of UCP expression vector

1.1

- 5 Human UCP3 cDNA was amplified by PCR using Pfu polymerase (Stratagene), from a cDNA comprising the polynucleotide sequence of SEQ ID NO:1 as template. The PCR product was sequenced and cloned into the EcoRV site of pBluescript (Stratagene) via blunt end ligation.

10 1.2

The human skeletal muscle specific alpha-actin promoter was excised from vector pACTSV40 (Fazeli et al (1996) J Cell Biol, 135 p241-251) as a 2.2 kb HindIII fragment, and cloned into the unique SmaI site of the vector from 1.1, again via blunt end ligation.

15 1.3

An artificial intron sequence was amplified by PCR using Pfu polymerase, with pIRES1neo (Clontech) as template and the following primer pair: 5' GCTGGAATTAATTCGCTGTCTGCGAG 3' and 5' ATGCATGCTCGACCTGCAGTTGGAACC 3'. The PCR fragment was then cloned into
20 the XhoI and SfiI sites of pCEP4 (Invitrogen), via blunt end ligation.

1.4.

The artificial intron-SV40 polyA cassette was excised from the vector of 1.3 as a Sall-XhoI fragment and cloned into the XhoI site of the vector of 1.2 downstream of the
25 human UCP3 cDNA.

1.5.

The transgene DNA was excised from the vector of 1.4 with KpnI and partial NotI restriction enzyme digests, gel-purified as a 3.9 kb DNA fragment, and injected into male
30 pronuclei of fertilised eggs.

Example 2 – Phenotypic effect of human UCP3 overexpression in transgenic mice

Male and female mice expressing human skeletal muscle UCP3 and age-matched wild-type C57Bl/6xCBA mice were housed in threes on a 12 h light cycle. Measurements of body weight and food consumption were commenced at 4 weeks until 12 weeks of age. All mice were fed TEK 2018 (TEKLAD) diet. Oral glucose tolerance tests were
5 performed at 8 and 12 weeks of age. Tail-tip blood was measured at times 0 and then 45, 90 and 135 min following an oral glucose (3 g/kg) load. Plasma glucose concentrations were determined and glucose disposal depicted as area under the glucose response curve with time.

Figure 1. shows the effect of overexpression of human UCP3 in mouse skeletal
10 muscle on body weight and 24h-food consumption. Both male and female UCP3 transgenic mice have reduced body weight with respect to age-matched wild type controls despite showing an increased 24h-food intake. Data from 10 – 12 animals (body weight) or 4 cages (n=3 per cage; food intake) per group, *P<0.05.

Figure 2. shows the effect of overexpression of human UCP3 in mouse skeletal
15 muscle on glucose disposal. Glucose disposal deduced from the area under the OGTT curve was greater in humanUCP3 transgenic mice compared to wild type mice. Data from 10 – 12 animals per group, *P<0.05.

All publications and references, including but not limited to patents and patent
20 applications, cited in this specification are herein incorporated by reference in their entirety as if each individual publication or reference were specifically and individually indicated to be incorporated by reference herein as being fully set forth. Any patent application to which this application claims priority is also incorporated by reference herein in its entirety in the manner described above for publications and references.

25

Claims

1. A transgenic rodent whose genome comprises a polynucleotide encoding a polypeptide which has at least 95% identity to the human mitochondrial uncoupling protein-3 (UCP3) of SEQ ID NO:2 under the control of a regulatory sequence facilitating expression of said polypeptide.
2. A transgenic rodent according to claim 1 wherein the rodent is selected from the group consisting of:
 - a) mouse; and
 - b) rat.
3. A transgenic rodent according to claim 1 wherein the polypeptide comprises a polypeptide having the amino acid sequence of SEQ ID NO:2
4. A transgenic rodent according to claim 3 wherein the polypeptide has the amino acid of SEQ ID NO:2.
5. A transgenic rodent according to any one of claims 1 to 4 wherein the polypeptide is encoded by a polynucleotide having at least 95% identity to the polynucleotide sequence of SEQ ID NO:1.
6. A transgenic rodent according to claim 5 wherein the polypeptide is encoded by the polynucleotide of SEQ ID NO:1.
7. The transgenic rodent of claim 1 wherein the UCP polypeptide is expressed predominantly in skeletal muscle, such expression being facilitated by a skeletal muscle-specific promoter.
8. The transgenic rodent of claim 7 wherein the skeletal muscle-specific promoter is alpha-actin.

9. The transgenic rodent of any one of claims 1 to 7 exhibiting reduced body weight.
 10. The transgenic rodent of any one of claims 1 to 7 exhibiting increased wound-
5 healing.
-
11. A method of producing the transgenic rodent of any one of the preceding claims comprising the steps:
 - 10 a) preparing transgene construct comprising coding region of the gene of interest operably linked to an appropriate regulatory sequence;
 - b) removing vector sequences by restriction digest;
 - c) introducing the transgene into the rodent by pronuclear injection; and
 - d) re-transferring the injected eggs into the uteri of pseudo-pregnant recipient mothers.
 - 15 12. A method of producing a transgenic rodent according to claim 11, wherein the rodent is a mouse and the transgene is introduced into mouse ES cells, using electroporation, retroviral vectors or lipofection for gene transfer.
 - 20 13. A transgene comprising a polynucleotide encoding the human UCP-3 polypeptide operably linked to a rodent regulatory sequence.
 14. A transgene according to claim 13 wherein the rodent regulatory sequence is the alpha-actin promoter.
 - 25 15. A method of testing a compound comprising exposing a transgenic rodent of any one of claims 1 to 10 to said compound and measuring phenotypic effects.

SEQUENCE LISTING

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P32426

Abstract

A transgenic mouse expressing human uncoupling protein 3 (UCP) is disclosed, together with methods of preparation and the uses thereof.

5

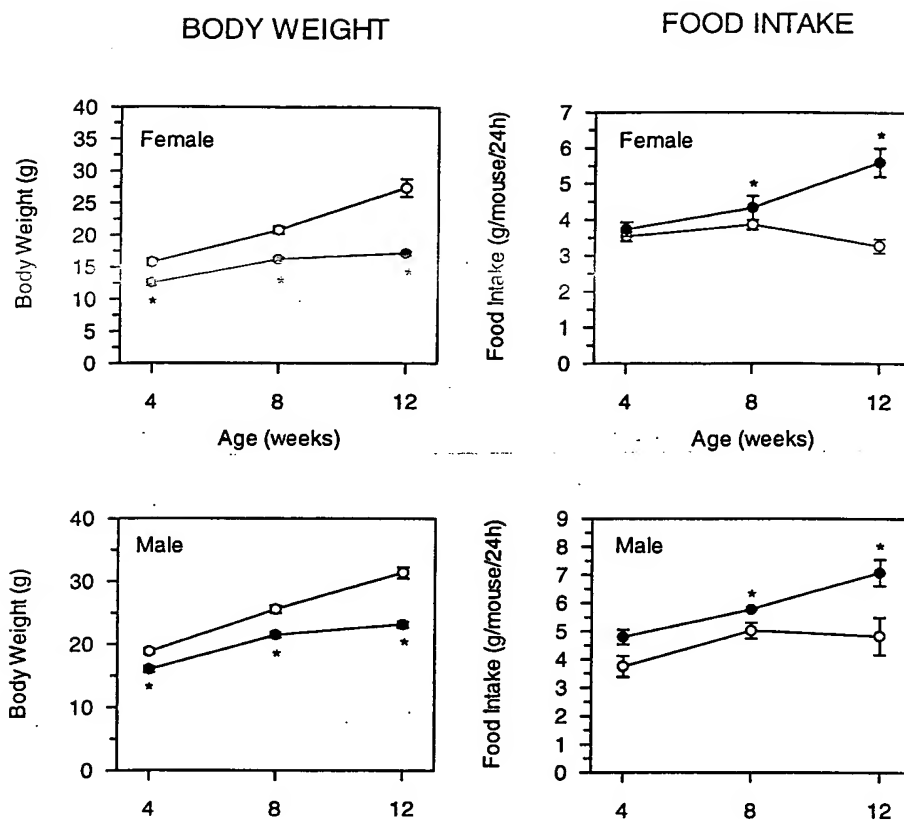


Figure 1

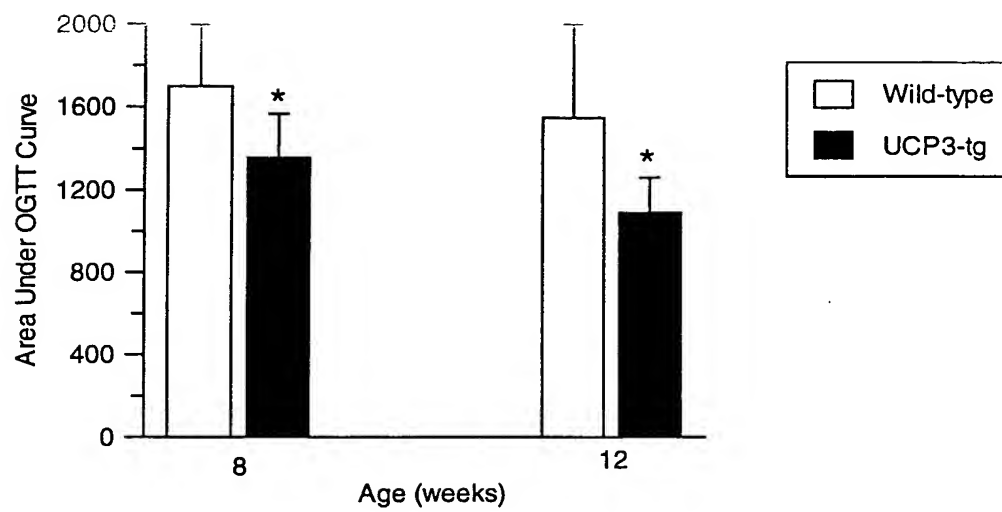


Figure 2